

An estimate of *Salmonella* prevalence on Illinois swine farms using mesenteric lymph node cultures

Damman D, Bahnson, P.B., Weigel, R.M., Isaacson, R.E., Troutt, H.F., and Kim, J.Y.

Department of Veterinary Clinical Medicine and Department of Veterinary Pathobiology
University of Illinois at Urbana-Champaign, Urbana IL, USA

Introduction

Accurate description of *Salmonella* prevalence is an important step toward understanding the epidemiology of the organism. A U.S. national prevalence estimate of fecal prevalence of *Salmonella* species showed that the organisms were commonly detected on farms, with 58 of 160 farms (38.2%) with one or more positive sample. (Anon. 1995a) The same survey, however, suggested that the prevalence rate differed by geographic region, with the southeastern area of the country having more than double the prevalence of the Midwest.

Since the presence of *Salmonella* in the gastro-intestinal tract provides direct evidence of shedding of *Salmonella* by pigs, it may be a useful predictor of the risk of pork contamination. Detection of *Salmonella* in herds where it may be present at a low prevalence necessitates the collection of a large number of samples. However, culture methods are relatively expensive and labor intensive. The detection of *Salmonella* in pooled samples has the potential to reduce costs and labor, and may be a useful tool to screen for shedding of *Salmonella* spp. However, culturing individuals produces a more accurate estimate of the within herd prevalence. Thus, culture based studies must seek to balance the trade-off between increased sensitivity to detect *Salmonella* herds and the accuracy of prevalence estimates within herds.

Lymph nodes become part of some pork products and act as a biological indicator of what a pig's exposure has been before slaughter. Thus, culture of lymph nodes may more directly reflect risk of pork contamination than would culture of fecal samples. Further, the expected higher detect rate for lymph nodes vs. fecal samples should result in increased sensitivity of detection.

This study was designed to identify efficient sampling methods, detect *Salmonella* spp. in pigs by bacterial culture, evaluate the effectiveness of pooling as a technique to increase sensitivity, and describe the number of herds with *Salmonella* detectable in slaughter weight pigs from Illinois.

Materials and Methods

Samples were collected from two large-scale abattoirs in Illinois. To be eligible, herds sold market weight pigs to the abattoirs participating in the study and had a history of delivering groups of at least 30 animals on a single day. A list of potential participants was constructed using a combination of abattoir records, the Illinois Pork Producers Association membership list, and client lists from swine veterinary practices and marketing groups. Three hundred and twenty-eight farms met the criteria for participation. Farms were asked to participate by a solicitation letter or by direct communication with plant employees. Samples were collected over a 14-month period from farms that delivered on days we were available to collect samples.

Caudal mesenteric lymph nodes were collected from 30 animals from each herd. Carcasses and viscera were marked during processing by matching the abattoir's identification number to the list of participating farms. Marked viscera were removed from the processing line, then dissected using clean dissection technique to harvest lymph nodes. Lymph nodes were individually packed in sterile plastic bags and transported to the laboratory on ice. Processing of all pooled samples was completed within 24 hours of collection. Two grams of lymph node from each of five individual pigs were then pooled to form six pools from each herd. The remaining lymph node tissue was stored at -70°C for further sampling. The pooled lymph nodes were homogenized then cultured. Samples were processed using a modification of an existing method. Briefly, initial enrichment in tetrathionate broth (48 hours at 37°C) followed by Rappaport broth (24 hours at 37°C). Samples were streaked onto XLT4 agar for isolation. Suspect colonies were re-streaked onto brilliant green agar to isolate pure colonies and to detect lactose fermentation ability. Genus level confirmation was by agglutination to polyvalent antiserum. After identification of positive pooled samples, frozen lymph nodes from positive pools were thawed and cultured using the same technique described for pooled samples.

Data were summarized to estimate the proportion of herds with culture positive samples pooled samples. The apparent within-herd pig prevalence was defined as the number of positive individual pigs, determined by culturing the

individuals within the positive pools, divided by the total number of pigs sampled in the herd. The relationship between pooled culture results and individual pigs culture results was determined by simple linear regression analysis. Data was transformed where appropriate to meet model-building assumptions.

Results

We sampled 142 of the 205 herds contacted that agreed to participate in this study. Pooled cultures were performed on all 142 herds while individual cultures were performed on 80 of the positive herds. We detected *Salmonella* in 96 of those herds for an overall prevalence of 67.6% (95% CI 64.0%-71.2%). Due to appropriation of funding for culturing the individual samples after the start of the study, we only had 80 herds available to compare individual positives to pool positives. A significant relationship ($p < .001$) existed between the number of positive pools and the number of individual pigs that were positive within a given marketing. (Figure 1) The coefficient of determination (R^2) was 0.48. In six of 80 herds, pools were positive but all individual pig cultures of frozen samples were negative. Herds with six, five, four, three, two, and one positive pool averaged 13.5, 6.1, 5.2, 2.9, 1.4, and .7 positive individual animals respectively.

Within all positive herds, there were 247 positive pools for an average of 2.5 positive pools per marketing. Within the 80 positive herds with individuals cultured, we were able to isolate *Salmonella* from 197 pools and 263 individual pigs. Thus, the apparent individual pig prevalence was 10.9 % (263/2400). The median prevalence for these herds was 6.67%.

The average number of animals marketed per farm per year was 9,235 representing a total of 905,057 market animals annually. The average number of sows for herds with this stage of production was 536, representing 50,881 breeding animals.

Discussion

The overall herd prevalence determined in this study is similar to that detected for herds in the United States and in other major pork producing countries. The apparent pig (individual) prevalence is somewhat higher than reported in previous studies. Two grams per individual was used in this study while others have used a one gram sample.^{4,5} The larger sample of lymph node used in this study may have enhanced sensitivity to detect positive pigs.

The final apparent individual pig prevalence may be underestimated, since we cultured lymph nodes from individual pigs only when a pooled sample was positive. Negative pools, are likely to contain some positive individual pigs as well and since individual lymph nodes from negative pools were not cultured, a few positive pigs surely have

been missed. However, the number of *Salmonella* positive pigs in a negative pool is probably small and may have little effect on the overall prevalence estimate.

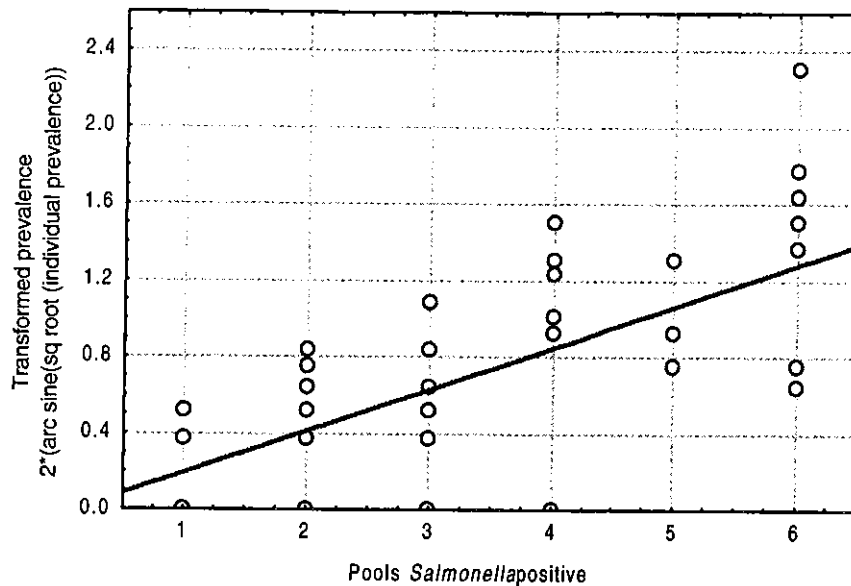
The selection of herds was not a random representation of all Illinois herds. Selection was limited to only two of three major slaughter plants in Illinois and those herds sampled were required to deliver a minimum of 30 pigs on a given day. In addition, we started with a sampling list constructed from several convenience sources. However, the herds sampled were from farms whose production represents nearly ten percent of the market swine herd within the state of Illinois according to the Illinois Department of Agriculture census.⁶

The majority of the herds were either negative or had only one positive pool, suggesting that most herds had a low prevalence of *Salmonella*. The discrepancy between mean (10.9 %) and median (6.7%) apparent individual pig prevalence suggests that a small proportion of herds had high apparent individual pig prevalence. This is similar to the pools prevalence distributions in that many herds are negative or low prevalence with a small proportion of high prevalence herds.

When the pooled samples were correlated with results from individual pigs, the transformed apparent individual pig prevalence increased as the number of positive pools increased. However, the number of positive pools represents only a portion of the variation in apparent individual pig prevalence, as suggested by the R^2 of 0.48. Thus, pooled samples crudely reflect the individual pig prevalence. Thus, pooled sampling may be justified in future studies if precise estimation of individual pig prevalence is not necessary.

Herds with three or more *Salmonella* positive pooled samples contributed a disproportionately high proportion of all culture-positive individuals detected, since their apparent individual pig prevalence was also correspondingly higher. These results suggest that pooled samples may be useful to identify herds with *Salmonella* that then could be targeted for intervention. Since a small number of marketing groups appear to contribute disproportionately to the prevalence of *Salmonella* at slaughter, it may be useful to target these source herds for follow up. If it was possible to identify specific control points in these herds, focus on a smaller number of herds may substantially reduce the effort required to reduce the prevalence of *Salmonella* positive pigs delivered for slaughter.

Figure 1. The relationship between number of pooled samples positive and transformed prevalence of *Salmonella* positive cultures among 61 herds.



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